UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

002581

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Evaluation of Toxicology Studies 743 744
Caswell No.'s: 56, 57, 59, 101, 221, ~~232~~, ~~233~~, 245, 253, 265, 501A

ID#61-172 Action Code: 400
Acc. #'s: 248738-248747

TO: Jacoby/Beavers, PM-21
Registration Division (TS-767c)

Studies submitted by Koppers Company, Inc. (under letter cover, October 20, 1982) have been evaluated, and are summarized on the following pages. DER's of the individual studies are also appended.

03/11/83

Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

1736

Active Ingredient	Caswell No.	Reg. No(s)	Study Title and Date	Accession No.	TB-Core Grade/ Evaluation
Arsenic acid	56	61-171	Evaluation of the Comparative Genotoxic Activities of Arsenic Acid in the Bacillus subtilis Differential DNA Repair Assay (June, 1981)	248742	ACCEPTABLE
			Evaluation of the Comparative Genotoxic Activities of Arsenic Acid in the E. coli WP_2, WP_2uvrA, and CM571 Tryptophan Reversion Assay. (June, 1981)	248743	UNACCEPTABLE
Copper naphthenate	245	61-172, 453-68, 453-283, 453-290, 453-282	Microbiological Genotoxicity Assays on Copper Naphthenate (January, 1981)	248738	Conditionally acceptable for Ames and E. coli; UNACCEPTABLE for yeast; ACCEPTABLE for DNA.
		453-290	Range Finding Toxicity Tests on Copper-treat 00 Concentrate (October, 1981)	248739	Oral, Dermal and Skin - SUPPLEMENTARY; Inhalation and Eye - INVALID
Copper-8-quinolinolate	253	453-281, 453-285, 453-286, 453-287, 453-289	Microbiological Genotoxicity Assays on Copper-8-Quinolinolate (January, 1981)	248746	Conditionally acceptable for Ames, E. coli; yeast - ACCEPTABLE; DNA - UNACCEPTABLE

Active Ingredient	Caswell No.	Reg. No(s)	Study Title and Date	Accession No.	TB-Core Grade/ Evaluation
3-Iodo-2 propynyl butyl carbamate (Troysan)	501A	453-284	Microbiological Genotoxicity Assays on Troysan (December, 1980)	248744	Conditionally acceptable for Ames and E. coli; yeast - UNACCEPTABLE; DNA-ACCEPTABLE
			Range Finding Toxicity tests on Woodtreat WB Concentrate (May, 1981)	248747	Oral, Dermal, Inhalation, Skin, Eye - SUPPLEMENTARY
			Range Finding Toxicity Tests on a Diluted Solution of Woodtreat WB Concentrate (May, 1981)	248740	Oral, Inhalation, Skin and Eye - INVALID; Dermal - SUPPLEMENTARY
Chromic acid Cupric oxide Arsenic pentoxide	221 265 57	61-128 61-128-453	Dermal Corrosivity Study of Wolmanac Concentrate 50% in Rabbits (August, 1976)	248741	Skin - MINIMUM Absorption - SUPPLEMENTARY Inhalation - INVALID
Tri-butyltin Oxide (TBTO)	101	453-272, 453-273, 453-274, 453-275, 453-276, 453-277, 453-278, 453-279,	Microbiological Genotoxicity Assays on Tributyltin Oxide (December, 1980)	248745	Conditionally acceptable for Ames, yeast; UNACCEPTABLE for E. coli and DNA.

Active Ingredient	Caswell No.	Reg. No(s)	Study Title and Date	Accession No.	TB-Core Grade/ Evaluation
Arsenic Trioxide	59	61-128, 61-170, 61-110, 61-141, 61-111, 61-124, 61-127, 61-139, 61-140, 61-128-453, 61-171, 61-134	Evaluation of the Comparative Genotoxic Activities of Arsenic Trioxide in the B. subtilis Differential DNA Repair Assay (June, 1981)	248742	ACCEPTABLE
			Evaluation of the Comparative Genotoxic Activities of Arsenic Trioxide in the E. coli WP_2, WP_2uvrA and CM571 Tryptophan reversion. (June, 1981)	248743	UNACCEPTABLE
Sodium Arsenite Sodium Arsenate	~~273~~ 744 ~~742~~ 743	61-128, 61-170, 61-110, 61-141, 61-111, 61-124, 61-127, 61-139, 61-140, 61-128-453, 61-171, 61-134	Evaluation of the Comparative Genotoxic Activities in the B. subtilis Differential DNA Repair Assay (June, 1981)	248742	ACCEPTABLE
			Evaluation of the Comparative Genotoxic Activities in the E. coli WP_2, WP_2uvrA and CM571 Tryptophan Reversion Assay. (June, 1981).	248743	UNACCEPTABLE

Copper naphthenate (Caswell No. 245) Acc. #248738

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(DER) DATA EVALUATION RECORD

FORMULATION: Not stated

CITATION: Microbiological genotoxicity assay of copper naphthenate, active ingredient of Copperteat®. (Study No. 3002-6, January, 1981, by Genex Corp., Rockville, MD; submitted to Koppers Co., Pittsburgh, PA.)

TRADE SECRET CLAIM: CBI

REVIEWED BY: Irving Mauer Ph.D.
HED/TB

Approved by:
(Date)

DATE OF REVIEW: 02/14/83

TEST TYPES: In vitro Mutagenicity assays: (A) Salmonella/microsome Assay (Ames); (B) Escherichia coli WP2 (uvr-A) Assay; (C) Saccharomyces cerevisiae D7 Assay; and (D) Differential DNA Repair Assay with Bacillus subtilis H17/M45.

STUDY A: Salmonella/Microsome (Ames) Assay.

Reversion to histidine independence (his+) was examined in replicate agar plate assays using five histidine-requiring (his-) auxotrophic strains (TA 1535, TA 1537, TA 1538, TA98, TA 100) of Salmonella typhimurium exposed to test chemical in ethanol at five concentrations ranging from 0.005 to 5.0 ul/plate (test-1), and 0.001 to 10.0 ul/plate (test-2), both in the absence as well as in the presence of a mammalian metabolic activation system provided by the S-9 microsomal fraction of liver prepared from Aroclor 1254-treated adult male rats, plus enzyme-generating co-factors. Negative controls (the diluent, ethanol) and appropriate positive controls (the mutagens: sodium azide, 9-aminoacridine and 2-nitrofluorene for non-activated assays; 2-anthramine [2-aminoanthracene] in both activated and non-activated assays) were included in each experiment. Duplicate plates were tested at each dose of test compound and both types of control.

The highest concentrations in each of the two tests (5 and 10 ul) were toxic. At no dose in either experiment, however, were any dose-related increases over solvent (ethanol) controls recorded in revertents to his+. Plates treated with the positive control substances (mutagens) responded as expected, inducing reversions 6 to 20 times solvent control.

The authors concluded that copper naphthenate was not mutagenic in the Salmonella/microsome (Ames) assay by the procedures employed in five indicator strains.

EVALUATION: The study would be ACCEPTABLE with registrant's provision of both the nature of the a.i. (technical?) and characteristics of test substance (purity, etc.).

STUDY 5: Escherichia coli WP_2 (uvrA) Assay.

Reversion to tryptophan-independence (tryp+), by either base pair substitution or foreward suppressor mutation, was examined in replicate agar plate assays using the tryptophan-requiring (tryp-) WP2 strain of E. coli, also deficient in DNA-damage repair (uvrA). Duplicate plates in each experiment were exposed to multiple (5 and 6) concentrations of test chemical spanning the ranges 0.005 to 5.0 ul/plate (test-1) and 0.001 to 10.0 ul/plate (test-2), both in the absence as well as in the presence of a mammalian metabolic activation system provided by the S-9 microsomal fraction of livers prepared from Aroclor 1254-treated adult male rats, plus enzyme-generating co-factors. Negative controls (the diluent, ethanol) and appropriate positive controls (the mutagens: N-methyl-N'-nitro-N-nitrosoguanidine [MNNG, 2.0 ug/plate] in non-activated assays; and 2-aminoanthracene

[2-ANTH, 10 ug/plate] for activated assays) were included in each experiment.

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The highest concentrations in each of the two tests (5 and 10 ul/plate) were toxic, both with and without the S-9 activation system. No increases over solvent control in revertents to tryp+ were observed at any concentration of test chemical, in contrast to responses by the positive controls, MNNG (5 times negative control) and 2-ANTH (10 times).

The authors concluded that copper naphthenate was not mutagenic in the E. coli WP2 assay by the procedures employed.

EVALUATION: The study would be ACCEPTABLE with registrant providing both the nature of the active ingredient (technical ?), as well as its purity and other physicochemical characteristics.

STUDY C: Saccharomyces cerevisiae D7 Assay.

Simultaneous detection of mitotic recombination (ade2+/- to ade2-/-), gene conversion (tryp- to tryp+), and reversion to isoleucine independence (iso- to iso+) were examined in aliquots from replicate suspension assays of the D7 strain of S. cerevisiae, exposed to test compound in ethanol at multiple concentrations (5 in test-1, 7 in test-2) ranging from 0.0001 to 0.01% (v/v), both in the absence of as well in the presence of a mammalian metabolic activation system provided by the S-9 microsomal fraction of livers prepared from Aroclor 1254-treated adult male rats, plus enzyme-generating co-factors. Negative controls (the diluent, ethanol) and positive controls (the mutagens: N-methyl-N'-nitro-N-nitrosoguanidine [0.0002 or 0.0005 % w/v MNNG] for the non-activated assays; and sterigmatocystin [0.075, 0.1 or 0.5 % w/v SC] for both types of assays) were included in each experiment. Triplicate plates were tested at each dose of test compound and both types of control.

In preliminary experiments without activation (data not shown), exposure to 0.005% (v/v) test chemical was lethal (0% survival), whereas 0.001% resulted in 100% survival, and 0.0025% left less than 10% survivors. A narrow toxicity range was also observed with the S-9 system (data not shown), namely at 0.01 and 0.03% (v/v).

The first set of experiments recorded moderate toxicity at the highest dose, 0.01% (57% survivors), and increases in both the absolute number of recombinants and number of recombinants per 10^5 survivors in the presence of S-9 (3.6

times the ethanol control), but no increases in tryp+ convertents, or iso+ revertents. The top dose, 0.0025% (v/v) was also toxic in the absence of S-9 (7% survival), and induced 1.5 X the number of recombinants (aberrant colonies); 1.9 X the number of tryp+ convertents, and 2.8 X the number of iso+ revertents. No differences from the ethanol control in any of the three genotoxic endpoints measured were recorded at any other dose of test substance.

In the second set of tests, toxicity was observed in the absence of S-9 at the two highest doses of 0.0020 and 0.0025% (v/v) test chemical (7% survival), and increases (twice or more negative control) recorded in number of aberrant colonies (recombinants) but not in tryp+ convertents or iso+ revertents. In activated cultures, 0.01% (v/v) was toxic (16% survivors), the two next highest concentrations, lethal, but no increases in any of the genotoxic endpoints observed at these or lesser doses.

A final experiment, to examine only recombination in the toxic range (0.001 to 0.01%, v/v), revealed increases in both absolute and relative numbers of recombinants in a non dose-related fashion, ranging from 1.3 to 3.6 times the ethanol control, at survivals of 50% or greater.

The authors concluded that a narrow range of toxic doses of copper naphthenate induced reproducible but not dose-related increases in the number of recombinants in two of the three experiments that yielded acceptable data, and thus may be considered a (weak) recombinogen i.e., induces DNA damage in this yeast system (S. cerevisiae D7).

EVALUATION: The design and conduct of this study were generally adequate, but the study as a whole is UNACCEPTABLE as a comprehensive assay, because of the following deficiencies:

1. Test compound was not characterized as to nature (technical ?), purity or other physicochemical characteristics.

2. In those tests involving activation (S-9), there were unsatisfactory responses to the positive control, sterigmatocystin. The single experiment at 0.5% (w/v) gave a response 50 times the negative control, but the responses at lesser concentrations (0.1 and 0.075 % w/v) were not different from the ethanol control in three of the four tests. This was in contrast to the response of non-activated cultures to MNNG, characteristically 20 to 100 times solvent

control culture values. A repeat of the activated assay is recommended, employing more consistently responsive positive control substances. 00:581

STUDY D: Differential DNA Repair Assay with Bacillus subtilis H17/M45.

Relative toxicities of test compound in deficient (M45, rec-) and proficient (H17, rec+) strains of B. subtilis were assayed in replicate disc tests , each in duplicate, at concentrations of 0.001 and 0.005 ml (test-1), and 0.001 and 0.01 ml (test-2). Kanamycin (0.1 mg) or commercial bleach (0.001 ml) served as negative controls, whereas the mutagens, ethylmethanesulfonate (EMS) or 1,2,3,4-diepoxybutane (DEB) were used as positive controls (at 0.001 ml each) (Activation tests were not included, since S-9 Mix does not function in these disc assays.)

Clear dose-response increases in the zones of growth inhibition (i.e., greater toxicity) were shown by the repair deficient strain over those of the proficient strain in both experiments. The negative controls gave equal zones of toxicities (i.e., not genotoxic), while the expected differential toxities were shown by the positive controls, EMS and DEB.

The authors concluded that copper naphthenate is positive in this assay, i.e., that it may interact with B. subtilis DNA, inducing damage.

EVALUATION: This study is ACCEPTABLE.

Copper naphthenate (Caswell No. 245) Acc. No. 248939

(DER) DATA EVALUATION RECORD 002581

FORMULATION: Copper Treat" 00 Concentrate, "a green viscous liquid" [no other characteristics stated].

CITATION: Range-Finding Toxicity Tests. (Report No. 44-530, October 26, 1981, from Bushy Run Research Center, Export, PA; sponsored by Koppers Co., Pittsburgh, PA.)

TRADE SECRET CLAIM: CBI

REVIEWED BY: Irving Mauer Ph.D. HED/TB APPROVED BY: (Date)

DATE OF REVIEW: 02/16/83

TEST TYPES: Acute Toxicity Tests: (A) Oral LD_{50} - Rat; (B) Acute Dermal LD_{50} Rabbit; (C) Acute Inhalation - Rat; (D) Acute Skin Irritation - Rabbit; (E) Acute Eye Irritation - Rabbit.

STUDIES:

A. Acute Oral Toxicity in Rats.

Young (3-4 wk) Wistar-derived male rats were given a geometric series of single doses of test compound by intubation (2,4, 8, and 16 ml/kg), observed for 14 days, and the LD_{50} calculated by the moving point average method. Death and toxic signs (distended abdomens, "sluggishness," tarry stools), 0 to 6 days after treatment, occurred at doses of 8 and 16 ml/kg, but none below 8 ml/kg; no weight loss was found in surviving animals. Calculated LD_{50} was 6.50 (4.80-8.79) ml/kg. (TOX. CAT. IV)

Gross pathological examination of dead rats revealed pale livers, stomachs filled with green liquid, and intestines injected (green in color); nothing remarkable was found in survivors.

EVALUATION: The study is CORE SUPPLEMENTARY because information on the test chemical was not given (purity, etc.).

B. Acute Dermal Toxicity in Rabbits.

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The trunk fur of New Zealand White males (3-5 months old) was clipped, and 8 or 16 ml/kg test material applied to intact skin and retained under impervious sheeting for 24 hours (when it was washed off to prevent ingestion). The LD_{50} was calculated as 16.0 (4.48-57.2) ml/kg (2 deaths/4 treated after 2 days at 16 ml/kg; 0/2 at 8 ml/kg). (TOX. CAT. III)

Moderate weight losses were observed, but no skin irritation or toxic signs. Gross pathological examination of dead animals revealed red urine in one, anuresis in the other, but nothing remarkable in survivors.

The study is CORE SUPPLEMENTARY, since information on the test chemical (purity, etc) was not given, nor was time of post-treatment observation explicitly stated.

C. Acute Inhalation in Rats.

Rats (strain and sex not explicitly stated) were exposed for 6 hours at room temperature to a "saturated vapor" of test compound generated over the previous 16 hours in a sealed Plexiglas chamber.

There were no deaths or weight losses among 6 animals exposed to these static conditions, but the following signs were noted at the end of the 6 hours exposure: tremors, lacrimation, dyspnea and pilerection. The $LT_{50} > 6$ hours.

The study is INVALID, because of the following deficiencies:

1. Information on test compound was not given.
2. Neither strain of sex of test animals was stated.
3. It was not stated how long animals were observed following removal from chamber.
4. Static exposure does meet EPA requirements; acute 4-hour dynamic exposure are presently required by "158" Rule-Making.
5. Actual concentration of test material was not given.

D. Acute Skin Irritation in Rabbits.

0.1 Ml of the (apparently) undiluted test material was applied to the clipped, uncovered, intact belly skin of 5

rabbits (sex and strain not explicitly stated), and the appearance of skin graded during a observation period of 24 hours. There was no irritation reported in 2 animals, moderate capillary injection in 2, and moderate erythema in 1. Grade 2 irritation was assigned by the authors. [TOX CAT. III, (minimum).]

This study is judged CORE-SUPPLEMENTARY, because:

(1) Information on test substance (purity, etc.) was not given.

(2) The animals were not observed for the standard 72-hour period.

(3) Neither strain nor strain of rabbit was given.

(4) It is not clear if "Standard Test Procedures" (submitted with study) were used, since it is stated elsewhere (page 4 of report) that: "Conditions - Standard. Applied undiluted."

E. Acute Eye Irritation in Rabbits.

Test material was instilled (presumably "undiluted", under "standard conditions" - p. 4 of report) into the conjunctival sacs of 5 rabbits, and eyes examined immediately, as well as graded after fluorescein staining at 24 hours, according to Standard Test Procedures appended to the report. No corneal injury was reportedly observed following instillation of 0.5 ml undiluted material. Grade 1 irritation (minimal) was assigned by the authors. [TOX. CAT. IV]

EVALUATION: This study is judged INVALID, because information on the test substance was not stated (purity, etc.), and an appropriate post-treatment period not observed (at least 7 days).

Troysan (Caswell No. 501A) Acc. No. 248740 002581

(DER) DATA EVALUATION RECORD

FORMULATION: Woodtreat WB Diluted Solution, "a white liquid" [no other characteristics stated].

CITATION: Range-Finding Toxicity Tests. [Report No. 44-512, May 11, 1981, Bushy Run Research Center, Export, PA; sponsored by Koppers Co., Pittsburgh, PA.]

TRADE SECRET CLAIM: CBI

REVIEWED BY: Irving Mauer Ph.D.
HED/TB

APPROVED BY:
(Date)

DATE OF REVIEW: 02/16/83

TEST TYPES: Acute Toxicity Tests: (A) Acute Oral LD_{50} - Rat. (B) Acute Dermal LD_{50} Rabbit; (C) Acute Inhalation LD_{50} - Rat; (D) Acute Skin Irritation - Rabbit; (E) Acute Eye Irritation - Rabbit.

STUDIES:

A. Acute Oral LD_{50} in Rats.

The report states that an LD_{50} > 16 ml/kg was found ("dosed as received"), according to "standard test procedures, attached" [However, no attachment was found in the submission.] A tabulation indicates that none of 6 animals died following 16 ml/kg, and normal weight gain was maintained. Salivation was observed at 5 minutes [how many animals, not stated], with recovery at 2 hours. It was stated that gross pathology revealed nothing remarkable, and it was concluded "...[test material] had an extremely low order of toxicity following acute peroral intubation." [Presumably, TOX. CAT. IV.].

EVALUATION: The study is judged INVALID, because no information on purity or other physicochemical characteristics of test substance was given, and no procedures included with this submission.

B. Acute Dermal LD_{50} in Rabbits.

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It is stated that the LD_{50} = 8.0 (4.90-13.1) ml/kg, "dosed as received" under "standard conditions" and "polyethylene sheeting" [However, no procedures are provided in this submission.] A tabulation lists a range of dosages and responses to support the calculated LD_{50}. Thus, at 2.0 and 4.0 ml/kg, there was no mortality (of 2 and 4 animals treated, respectively); whereas weight gain appeared to be normal [by 14 days, presumably, but period of observation was not separately stated], edema and ecchymosis (and desquamation at 4 ml/kg) were observed. At 8.0 ml/kg, 2 of 4 rats died (within 2 days), weight loss was recorded in survivors, who manifested epidermal desquamation and fissuring at 14 days; all 4 animals died following 16 ml/kg (in from 1 to 4 days), and edema and ecchymosis observed in all prior to death. Gross pathological examination among rats which died revealed red, mottled livers, dark spleens and reddish kidneys, but no changes were found in survivors. The authors concluded the test material was "slightly toxic following acute (covered) percutaneous application." [TOX. CAT. III]

EVALUATION: The study is judged CORE - SUPPLEMENTARY, since pharmaceutical information on the test substance, as well as further details on test protocol are required [not included in this submission].

C. Acute Inhalation in Rats.

Six rats [sex and strain not explicitly stated] were [presumably] exposed by static conditions at room temperature for 6 hours to a "substantially saturated vapor" of test substance [other conditions not stated], according to "Procedure B of standard test procedures" [not included in this submission]. No animals died, weight gain was low but not unusual [however, period of observation was not stated], and lacrimation and ataxia [severity not stated] observed "within 4 hours." Gross pathology was negative. The authors concluded the LT_{50} > 6.0 hours. [No TOX. CAT. can be ascertained from these data.]

EVALUATION: The study is judged CORE-INVALID, since many details on procedure are missing, and static exposure does not meet EPA requirements (Rule-Making, section 158, requires 4 hours dynamic exposure).

D. Acute Skin Irritation in Rabbits.

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Uncovered skin of 5 rabbits was anointed with undiluted test compound under "standard conditions" [not stated]. The stated results were: "No irritation on one rabbit, moderate capillary injection on 2, marked capillary injection on 2. Grade 3." [TOX. CAT. II-III]

EVALUATION: This report is judged CORE INVALID, since no informaton on test substance or protocol was included in submission.

E. Acute Eye Irritation in Rabbits.

Undiluted test compound (0.5 ml), instilled into the eyes of 5 rabbits [sex and strain not stated], was reported to cause no injury. Grade 1. [TOX. CAT. IV]

The study is INVALID, because there is no informaton on test substance and protocol; further, the period of post-treatment observation was not given [at least 7 days required].

WOLMANAC (chromic acid - Caswell No. 221; cupric oxide - Caswell No. 265; arsenic pentoxide - Caswell No. 57)

Acc. # 248012581

(DER) DATA EVALUATION AND RECORD

FORMULATION: Wolmanac Concentrate 50%, "a liquid." [No statement of formula included in submission.]

CITATIONS: (A) Dermal Corrosivity Study of Wolmanac Concentrate 50% in Rabbits. (Report No. 6E-2976, August 26, 1976, Cannon Labs. Inc., Reading, PA; submitted to Association of American Railroads, Edison, N.J.)

(B) Skin Absorption Study of Wolmanac Concentrate 50% on New Zealand Albino Rabbits. (Report No. 6E-2907, August 19, 1976, Cannon Labs, Reading, PA; submitted to Association of American Railroads, Edison, N.J.)

(C) Inhalation Class B Poison (Rat). (Report No. 63-2906, August 26, 1976, Cannon Lab., Reading, PA; submitted to Association of American Railroads, Edison, N.J.)

REVIEWED BY: Irving Mauer Ph.D.
HED/TB

APPROVED BY:
(Date)

DATE OF REVIEW: 02/17/83

TEST TYPES: (A) Skin Irritation - Rabbit (according to 16 CFR 1500.41/49 CFR 173.240). (B) Skin Absorption -Rabbit. (C) Acute Inhalation - Rat (according to Dept. Transportation, Tariff No. 25, April 24, 1972).

STUDIES:

A. Skin Irritation in Rabbits. The dorsal and flank fur of 6 New Zealand White rabbits was clipped, 0.5 ml of test material applied to each of 2 intact skin sites per animal under 1-in^2 gauze patches, covered with 2-in^2 plastic sheets secured with adhesive tape, and held in place for 4 hours (49 CFR 173.240) by impervious plastic sheeting around the entire trunk of each animal. At the end of the exposure period, all wrappings and patches were removed and the skin sites scored immediately, as well as 24 and 48 hours later, for edema, erythema and eschar formation, as well as for ulceration, necrosis or other irreversible tissue damage (16 CFR 1500.41). Three animals were dead by the 48-hours observation period. No signs of tissue destruction were noted in any of the 12 test sites at 4 or 24 hours, nor in the remaining animals at 48 hours. PIS = 1.00 for edema at 4 hours, 1.00 for both erythema and edema at both 24-hours and 48-hours observations. [TOX. CAT. III-IV]

EVALUATION: The study is judged CORE-MINIMUM.

B. Skin Absorption in Rabbits. 200 Mg/kg of test material was applied to the intact dorsal skin of 12 New Zealand White rabbits clipped free of fur, and held in place by elastic sleeves for 24 hours, at which time the sleeves were removed, and treated areas wiped clean of remaining excess material. The animals were observed for toxic signs and survival for 48 hours. All 12 animals died within 24 hours, with evidence of severe diarrhea. The authors concluded the test material was toxic at the dosage applied. [TOX. CAT. I]

EVALUATION: The study is judged CORE-SUPPLEMENTARY, since only one (lethal) dose was used, and no attempt made at deriving a NOEL.

C. Acute Inhalation in Rats. Ten young male rats (200-300 gm each) [strain not specified] were exposed for one hour to a vapor mist generated by bubbling houseline air at 5 L/min through the test material into a 40-liter chamber containing the animals. The nominal concentration calculated was 26.91 mg/l, based on the total amount of material used (8,072 mg) and the total air flow (volume of 300 l). Survivors of the 1-hr exposure were observed for 48 hours. No animals died, and there were no adverse signs or symptoms reported either during or after the exposure. The authors concluded that the test material was not a class B Poison according to the DOT (Tariff No. 25, April 24, 1972).

EVALUATION: The study is judged INVALID, since there is no assurance any of the test material was generated as an effective atmosphere; and were it so, static tests such as this do not meet Agency guidelines (Section 158, Rule-making).

Arsenic acid (Caswell No. 56) Acc. # 248742
Arsenic trioxide (Caswell No. 59)
Sodium arsenite (Caswell No. ~~233~~ 741
Sodium arsenate (Caswell No. ~~232~~ 743

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(DER) DATA EVALUATION RECORD

FORMULATIONS: "Samples No. 938-1A, 938-1B, 938-1C, and 938-1D, as 0.05 M solutions in water. [No other information on test chemicals was provided.]

CITATION: Evaluation of the Comparative Genotoxic Activities [of four arsenicals] in the Bacillus subtilis differential DNA Repair Assay. (Report No. 3002-8, June 1981, Genex Corp., Rockville, MD; sponsored by Koppers Co., Pittsburgh, PA.)

REVIEWED BY: Irving Mauer, Ph.D. APPROVED BY:
HED/TB (Date)

DATE OF REVIEW: 02/18/83

TEST TYPE: Mutagenicity--Repair Assay in Bacillus subtilis (H17/M45)

STUDY: Duplicate cultures of the repair-deficient strain M45 of B. subtilis (rec-) and its repair-proficient parental strain H17 (rec+) were exposed to paper discs soaked with 50 ul aliquots of test substances at each of three concentrations: 0.0125 M, 0.025 M and 0.05 M in two separate experiments, and zones of inhibition (distance in millimeters from disc edge to streaked bacteria) measured after 24 hour incubation. Solvent (sterile, deionized water), negative (0.5 mg. kanamycin), and positive (DEB, 0.005 ml 1,2,3,4-diepoxybutane) were included in each experiment.

No growth inhibition was observed in either tester strain (in both tests) to any of the test compounds (i.e., no toxicity), whereas the control substances produced the expected responses (equal zones in strains H17 and M45 for kananycin; 2 to 6 times the inhibitory zones in rec- compared to rec+ cultures for DEB). The authors concluded the assay was inconclusive ("no test"), since there was no toxicity to either strain by any of the arsenicals tested.

This study and its conclusion are ACCEPTABLE. ✓

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Arsenic acid (Caswell #56)
Arsenic trioxide (Caswell #59)
Sodium arsenite (~~Caswell #233~~) 144
Sodium arsenate (~~Caswell #232~~) 733

Acc. #248743

(DER) DATA EVALUATION RECORD

FORMULATIONS: "Samples No. 938-1A, 938-1B, 938-1C, and 938-1D, as 0.05 M solutions in water. [No other information on test substances was provided.]

CITATION: Evaluation of the Comparative Genotoxic Activities [of four arsenicals] in the E. coli WP_2, WP_2uvrA and CM571 Tryptophan Reversion Assay. (Report No. 3002-9, June 1981, Genex Corp., Rockville, MD; sponsored by Koppers Co., Pittsburgh, PA.)

REVIEWED BY: Irving Mauer, Ph.D. HED/TB
APPROVED BY: (Date)

DATE OF REVIEW: 02/18/83

TEST TYPE: Mutagenicity--Reverse gene (point) mutation in strains of Escherichia coli.

STUDY: Following preliminary dose range-finding toxicity tests, duplicate cultures of three auxotrophic tryptophan-requiring (tryp-) E. coli strains (WP2; WP2-uvrA, also deficient in excision repair; and CM571-rec A, deficient in recombination repair) were exposed in replicate experiments (on separate days with each strain) for either 15 minutes or one hour to four doses of each test substance (ranging from 2.0 to 125 X 10-4 M), both in the absence and presence of a mammalian metabolic activation system provided by the microsomal (S-9) fraction of livers from adult male Fischer 344 rats stimulated by Aroclor 1254 injection, plus enzyme-generating co-factors (S-9 Mix). Both negative (DW) and positive controls were included in each experiment: The mutagens, N-methyl-N'-nitroN-nitrosoguanidine (MNNG, at 1.36 X 10^{-4} M) for non-activated cultures; and 2-aminoanthracene (2-ANTH, at 2.07 X 10^{-4} M) with S-9 Mix. After exposure to test compounds, cultures were resuspended in buffer solution, incubated for two days, at which time colonies were counted; the number of tryp+ revertent colonies was calculated per 10^8 survivors.

A total of seven experiments were performed with all the test substances: Three using the WP2 strain, and two each using the uvrA and CM571 rec-strains. No toxicity and no

increase in tryp+ revertents were revealed when WP2 was treated for the conventional 15 minutes at dosages ranging up to 125 X 10^{-4} M (Tables 2 and 3 of report).

When the duration of exposure was increased to one hour, howerver, the top dose of 125 X 10^{-4} M arsenic acid was toxic (17% survival compared to water control), but only in the absence of S-9 Mix, and induced 3 times the frequency of revertents, an apparently positive result "...not confirmed by a repeat experiment with several doses because of time constraints of the study...." [authors' statement, report page 7]. None of the other test chemicals was toxic, nor increased reversion to tryp+ (Table 4).

Tests with the WP2-uvrA strain exposed for the two time periods at the same dose range (2 to 125 X 10^{-4} M) reported moderate degrees of toxicity (50-70% relative survival) at the higher concentrations (62.5 and 125 X 10^{-4}) accompanied by isolated small to moderate non-dose-related increases in revertents (ca. twice the water control), for both arsenic acid and arsenic trioxide, but not sodium arsenite or arsenate, and mainly in cultures without the metabolic activation system, S-9 Mix (Tables 5 and 6).

With 15-minutes exposure to doses up to 125 X 10^{-4} M, the CM571-recA strain was reported to respond no differently than the water controls [NB: This could not be verified, however, since the portion of Table 7 containing the control values as well as responses to arsenic trioxide and arsenic acid, which would have been p. 23 of the report, was missing from this review copy.] One hours' exposure, on the other hand, induced isolated toxicities of approximately 60% relative survival for both the trioxide as well as sodium arsenite, as well as moderate increases in revertent frequencies, but in a random fashion, e.g., 2.5-2.8 times control at the 2 and 20 X 10^{-4} M doses, but less than twice at higher doses (as shown in Table 8). [It is stated in the report text (p. 9) that arsenic trioxide and sodium arsenite were "...somewhat toxic, (but) there were no dose-related increases in revertents."]

The positive control for the non-activated assays (MNNG) gave the expected response, inducing from 50 to 1400 times the water control frequencies of tryp+ revertents. The report stated that: "The 15 minute and 1 hour exposures were not always sufficient to activate the S-9 positive control (2-anthramine)."

Since the chemicals were tested "...to the highest concentrations possible using the 0.05 M solutions provided by Koppers Co.,... and found to be not very toxic (except

for arsenic acid in one experiment), the authors stated that "...the results are inconclusive,..." and concluded that "...these compounds are nonmutagenic."

EVALUATION: The data submitted are considered inconclusive, and the study UNACCEPTABLE as a comprehensive assay for these arsenicals because of the following deficiencies:

1. The test materials were not adequately characterized.

2. The "highest dose possible" (p. 7) was not tested, since the sponsor provided 0.05 M solutions (= 500 x 10^{-4} M, i.e., "neat") and the top dose employed was only 25% of that (=125 x 10^{-4} M). The toxicity test was also inadequate for range-finding, since the top dose there was only 2 x 10^{-4} M, and produced no toxicity.

3. The definite positive response (3X control) registered in the (non-activated) WP2 strain (60 minute exposure to 125 X 10^{-4} M) was not confirmed, and further, an activation counterpart test (with S-9 Mix) was not conducted.

4. Whereas the non-activated tests were adequately controlled with the use of the mutagen, MNNG, the response of S-9 bolstered cultures to 2-ANTH was satisfactory in only two tests (barely in one, 3.9 X negative control), whereas in the remaining tests induced revertent rates less than twice water control, and in one case less than the response with test compound (with 60 minute exposure of strain CM 571).

Therefore the conclusion that "these compounds are nonmutagenic" is not warranted from the inadequate data reported in this study.

002581

Troysan (Caswell No. 501A) Acc. # 248744

(DER) DATA EVALUATION RECORD

FORMULATION: 3-iodo-2-propynyl butyl carbamate, "active ingredient in TROYSAN ®" [No other characterization given, but presumably received as a liquid according to dosage selection.]

CITATION: Microbiological Genotoxicity Assays on Troysan. (Report No. 3002-4, December, 1980, Genex Corp., Rockville, MD; sponsored by Koppers Co., Pittsburgh, PA.)
TRADE SECRET CLAIM: CBI

REVIEWED BY: Irving Mauer Ph.D. (HED/TB) APPROVED BY: (Date)

DATE OF REVIEW: February 23, 1983

TEST TYPES: Mutagenicity Assays--(A) Reverse mutation in Salmonella typhimurium strains (Ames Salmonella/microsome Assay); (B) Reverse mutation in Escherichia coli WP2 (uvrA); (C) Simultaneous detection of mitotic recombination and gene conversion (both DNA damage/repair assays), and reversion to isoleucine independence in Saccharomyces cerevisiae D7; (D) Differential DNA repair (toxicity) with Bacillus subtilis H17/M45.

STUDIES: (A) Ames Salmonella/microsome Assay.

Duplicate plate cultures of five histidine - requiring (his-) strains of S. typhimurium (TA 1535, TA 1537 TA 1538, TA 98, TA 100) were exposed to multiple doses of test substance ranging from 0.0005 to 5.0 ul/plate (5 concentrations in each of two replicate experiments), both in the absence and presence of the microsomal fraction (S-9) prepared from livers of Aroclor 1254-treated adult male SD rats (plus appropriate enzyme-generating cofactors), and colonies reverting to histidine-independence (his+ revertents) counted after 3 days' incubation. Both solvent (dimethylsulfoxide, DMSO) and positive controls appropriate to the bacterial strains and mode of activation (w/w'out S-9) were included in each experiment. (For unactivated assays: with TA 1535 and TA 100, 1.0 ug/plate sodium azide, with TA 1537, 50.0 ug/plate 9-aminoacridine, and with TA 1538 and TA98, 2-nitrofluorene; for all activation assays, 2.5 ug/plate 2-aminoanthracine with all 5 strains).

Concentrations at or above 0.5 ul test compound were found to be "toxic to all strains" [however, no data on

relative survivals were presented]; no dose-related increases in the number of his+ revertents over DMSO controls were observed in either experiment at any concentration, either in the presence or absence of S-9. Responses to the positive control agents were as expected, 10 to 20 times solvent controls (Tables 1 & 2 of report).

The authors concluded that 3-iodo-2-propynyl butyl carbamate (active ingredient of Troysan) was "...not mutagenic in this assay by the procedures [we have] used."

EVALUATION:

The study would be ACCEPTABLE, and the conclusions supported by valid data generated by adequate procedures, with the provision of test compound characterization (if technical grade, purity, etc.), and survival data at "toxic" doses.

B. Escherichia coli WP 2(uvrA) Assay

Duplicate plate cultures of the tryptophan-requiring (tryp-) WP2 strain of E. coli (also deficient in excision repair, hence uvr-) were exposed to 5 concentrations of test material ranging from 0.0001 to 2.5 ul/plate, both in the absence and presence of microsomes (S-9) from livers of Aroclor 1254-stimulated rats (as for the Ames Assay, above), and revertents to tryptophan-independence (tryp+) counted after 3 days' incubation. Positive controls (the mutagens: MNNG, 2 ug/plate, for non-activated cultures; 2-aminoanthracene, 10 ug/plate, with and without activation) and solvent (DMSO and S-9) controls were included in each experiment.

Test compound was reported to have been "slightly toxic" at 0.5 ul/plate (the top dose) in the first experiment, and "toxic" at 0.5 and 2.5 ul/ plate (the top dose) in the second test [but no data on relative survivals were presented]. No dose-related increases in tryp+ revertent colonies over solvent controls are indicated in tabulated data at any concentration of test material tested, in contrast to the expected responses to the positive controls, 6 to 10 times DMSO values (Tables 3 and : of report).

The authors included that 3-iodo-2-propynyl butyl carbamate (a.i. of Troysan) was "...not mutagenic in this assay by the procedures [we have] used."

EVALUATION: The study would be ACCEPTABLE, and the conclusions supported by valid data generated by adequate procedures, with the provision of test compound characterization (if

technical grade, purity, etc.), and survival data at "toxic" doses.

C. Saccharomyces cerevisiae D7 Assay.

Simultaneous detection of induced mitotic recombination (aberrant colonies), gene conversion (from tryptophan-requiring, tryp-, to independence, tryp+), and reversion to isoleucine prototrophy (from iso- to iso+) was assayed in the diploid yeast strain, D7, of S. cerevisiae treated for 4 hours, in each of three separate experiments with test compound at multiple concentrations ranging from 0.0001 to 0.5% (v/v), (each dose at least in triplicate), both without and in the presence of liver microsomes (S-9 plus co-factors) from Aroclor 1254-treated adult Sprague-Dawley male rats. Solvent controls (DMSO) as well as a positive mutagen for non-activated cultures (MNNG, 0.0006%, w/v) and for activation (sterigmatocystin, 0.05, 0.075 or 0.1% v/v) were included in each experiment.

The first experiment (5 doses, from 0.0005 to 0.5% v/v) was stated to yield no growth (i.e., 100% toxicity) at the two highest doses 0.05 and 0.5%. The two lower were not toxic, and slight elevations in the number of recombinants (1.3 to 1.4 times solvent control) were induced, but only in non-activated cultures. No increases were recorded in tryp+ convertents or in iso+ revertents at these doses (Tables 5 to 7, Summary Table 8).

The seven doses in the second experiment spanned the range 0.0001 to 0.03%. The highest was lethal, while 0.02% was moderate toxic, yielding 31% survivors relative to negative control in the absence of S-9, and 62% relative survival with S-9. In non-activated cultures, non-dose related increases in aberrant colonies (=recombinants) were observed at all doses (1.9 to 5.7 times control), but only at the higher, moderately toxic dose in S-9 supplemented cultures (3.2 X DMSO). No significant increases in tryp+ convertents or iso+ reversions were recorded in activated (S-9 cultures) although a slight increase (1.6x control) was induced in gene conversion at the toxic 0.02% dose (Tables 9 to 11, Summary Table 12).

In the final experiment (7 doses ranging in smaller increments from 0.0001 through 0.025%), the 0.02% and 0.025% doses were likewise toxic in nonactivated cultures (40% and 1% ~~colective~~ relative survival, respectively), but not in the presence of S-9. Despite the apparently anomalous fluctuations in toxicity, the test chemical induced increases in recombinants (1.4 to 8.5 X DMSO control), both with and without activation, but only in an "approximately" dose-related fashion. As in

test-2, no dose-related increases in tryp+ convertent or iso+ revertent colonies were observed, although the 0.02% dose (40% survival), without S-9, was stated to induce a small increment (1.9 X control) in convertents (Tables 13 to 15, Summary Table 16). [N.B.: As well, 2.2X control increase is recorded for iso+ revertents at this moderately toxic dose, as shown in Table 15.]

The authors concluded that 3-iodo-2-propynyl butyl carbamate (a.i. of Troysan) is "apparently recombinogenic," inducing increases in aberrant colonies, in the absence as well as in the presence of metabolic activation (S-9), but did not induce (determinative) dose-related increases in gene conversion (tryp+) or reverse gene mutation (iso+)

EVALUTION: Whereas the data presented and the conclusions drawn for the recombination position of these tests would be ACCEPTABLE with the provision of information on the test substance (purity, etc); the portions testing for gene conversion and reverse mutation are incomplete, and thus UNACCEPTABLE. The activation assays were (inexplicably) poorly controlled with the use of sterigmatocystin, at 0.05, 0.075 or 0.1%, since the response was most often less than twice the DMSO control (if not the same as control)

D. Differential DNA Repair Assay with Bacillus Subtilis H17/M45("Rec" Assay).

Duplicate cultures of the recombination repair strain M45 (rec-) and its parental proficient strain H17 (rec+) were exposed to paper discs soaked with test chemical (0.001 or 0.01 ml), and the diameter of zones of inhibition measured after 16 to 17 hours incubation in replicate tests. The genotoxic mutagen, 1,2,3,4,-diepoxybutane (DEB, 0.001 ml) served as positve control, and commercial bleach (0.01 ml) as negative control (produces toxicity only). [Metabolic activation is inappropriate in this type of assay.]

Both concentrations of test chemical produced equal zones of inhibition in both strains, and the authors concluded that no DNA damage was induced by (their) procedures used in this assay.

EVALUATION: The study is ACCEPTABLE.

002581

Tri-butyltin oxide (TBTO) (Caswell No. 101) Acc. # 248745

(DER) DATA EVALUATION RECORD

FORMULATION: Bis (tri-N-butyltin) oxide, "active ingredient in Tribucide®" [presumably a liquid, but no other information provided].

CITATION: Microbiological Genotoxicity Assays of Tributyltin Oxide. (Report No. 3002-5, December, 1980, Genex Corp., Rockville, MD; sponsored by Koppers Co., Pittsburgh, PA.)

TRADE SECRET CLAIM: CBI

REVIEWED BY: Irving Mauer, Ph.D. (HED/TB) Approved by:_____ (Date):_____

DATE OF REVIEW: February 25, 1983

TEST TYPES: Microbial mutagenicity Assays: (A) Reverse mutation in the Salmonella/microsome (Ames) Assay; (B) Escherichia coli WP2 (uvrA) Assay; (C) Mitotic recombination, gene conversion and reverse mutation in Saccharomyces cerevisiae D7; (D) Differential DNA repair (toxicity) assay in Bacillus subtilis H17/M45.

STUDIES:

A. Salmonella/microsome (Ames) Assay.

Duplicate cultures of five hisidine-requiring (his-) strains of S. typhimurium (TA 1535, TA 1537, TA 1538, TA 98 and TA 100) were exposed to five graded concentrations of test compound (dissolved in dimethylsulfoxide, DMSO) ranging from 0.0001 to 0.05 ul per plate, in replicate experiments, both in the absence and presence of a mammalian metabolic activation system provided by the microsomal fraction (S-9) prepared from the livers of Aroclor 1254-induced adult male Sprague-Dawley rats, plus cofactors (S-9 Mix). Reversions to histidine-independent (his+) colonies were enumerated after three days' incubation. A solvent control (DMSO), and positive controls appropriate to the bacterial strains and mode of testing were included in each experiment: The mutagens 2.5 ug/plate 2-anthramine for all strains in both activation and non-activated cultures; and in non-activated tests, 1.0 ug sodium azide for TA 1535 and TA 100; 50 ug 9-aminoacridine for TA 1537; and 5.0 ug 2-nitrofluorene for TA 1538 and TA 98 .

In both tests, the highest dose (0.05 ul) was reportedly "toxic" to all five strains, both with and without S-9 Mix, while the second highest, 0.005 ul [misstated in the text as "0.0005" ul] was also "somewhat toxic" [% survivals, however, were not presented]. No dose-related increases in histidine independent (his+) revertents were reported at any dosage in either experiment [although Table 1 and 2 record isolated small increases, 1.5 or less of the DMSO control in a few instances at some concentrations]. The expected responses to the positive controls substances were observed in all strains.

The authors concluded that bis (tri-N-butyltin) oxide (TBTO) was not mutagenic under the conditions used in this assay.

EVALUATION: The study would be ACCEPTABLE with the provision of information on the test substance (purity, if technical grade, etc.), as well as data on toxicity (% survivals).

B. Escherichia coli WP2 (uvrA) Assay.

Duplicate cultures of the tryptophan-requiring (tryp-) WP2 strain of E. coli, which is also deficient in excision repair (uvr-), were exposed to five graded concentrations of test substance ranging from 0.0001 to 0.05 ul/plate in two separate experiments, both with and without a mammalian metabolic activation system provided by the microsomal fraction (S-9) prepared from Aroclor 1254-induced adult male S-D rats, plus enzyme-generating cofactors. After incubation for three days, revertents to tryptophan-independence (tryp+ colonies), either by reverse or suppressor mutation, were counted. Negative and S-9 Mix control cultures (the solvent, DMSO) were included in each experiment, as well as the mutagens, N-methyl-N^1-nitro-nitrosoguanidine (MNNG, 2 ug/plate) for non-activated tests; and 2-aminoanthracene (2-ANTH, 10 ug/plate) for the activated assays.

The high dose was considered "somewhat toxic" in both experiments, both with and without the S-9 Mix [data on % survival, however, were not reported]. No increases in tryp+ revertents were recorded at any concentration [i.e., apparently not mutagenic in this study], in contrast to the expected increases in response to both positive controls (Tables 3 and 4).

EVALUATION: The study is faulted by not providing survival data at concentrations including "some[what] toxicity," suggesting inadequate testing. Further, information on the nature and purity of the test substance is required. The study is thus UNACCEPTABLE as reported.

C. Saccharomyces cerevisiae D7 Assay.

Triplicate (or more) cultures of the S. cerevisiae diploid strain D7 (yeast) were assayed for induced mitotic recombination (aberrant colonies), gene conversion (from tryptophan auxotrophy, tryp-, to independence, tryp+) and reversion to isoleucine independence (iso- to iso+, by either reverse mutation, or non-specific suppressor mutation), following 4 hours' incubation at a wide range of test compound concentrations, from 0.0000005 to 5.0% (v/v), in four replicate experiments performed on separate days, both in the absence and presence of microsomes prepared from the S-9 fraction of liver homogenates from Aroclor 1254-treated adult male Sprague-Dawley rats (S-9 Mix). Positive controls (the mutagens: MNNG at 0.002, 0.005 or 0.006% w/v, for non-activated tests; sterigmatocystin, 0.025, 0.05 or 0.1% w/v for activation) and solvent (DMSO, with and without S-9 mix) controls were included in each experiment.

The test substance was highly toxic to yeast cells at doses of 0.005% (v/v), leaving only 5-6% survivors both with and without S-9 Mix; it was also reported to be moderately toxic (36% survivors) at one-tenth that concentration (0.0005%), but only in the absence of the activation system. Although doses below this level were "somewhat" toxic (20-40% survivors) in some experiments, no dose-related increases in any of the three genotoxic end-points (recombination, gene conversion, or reversion to iso+) were reported in the first three experiments [although Tables 5 through 12 recorded a few isolated, small increases, 1.5 and less times DMSO controls]. [NB: Whereas response to the mutagen MNNG in non-activated tests was consistently good, that of 0.05% w/v sterigmatocystin and less was variable, most often no different from solvent controls.]

The final experiment assayed only the recombinogenic potential of the test compound in a narrow dose range (7 concentrations, from 0.0001 to 0.0025% v/v). Toxicity was observed at 0.0005% and greater, but no dose-related increases in aberrant colonies over controls reported [although Table 13 records isolated, small increases, 1.6 X DMSO control]. In this test, both positive controls (especially sterigmatocystin at 0.1%) performed as expected.

The authors concluded that bis (tri-N-butyltin) oxide "did not induce recombination, gene conversion, or reverse mutation in this assay system by the procedures [we have] used."

EVALUATION: The portion of this study testing for the detection of recombination can be considered ACCEPTABLE, whereas those segments reporting on gene conversion and reversion to isoleucine prototrophy are inconclusive, in the main due to inadequate response to (possibly) insufficient dosage of the positive control, sterigmatocystin. Additionally, as with the other studies in this series, details on the nature (technical grade?) and purity of the test material is lacking.

D. Differential DNA Repair (Toxicity) Assay with Bacillus Subtilis H17/M45.

Zones of growth inhibition in duplicate cultures of the recombination-repair deficient E. coli strain, M45 (rec-) and its repair-proficient parent, H17 (rec+) were compared in response to paper discs soaked with a "solution" [concentration unspecified] of test substance, 0.001 ml and 0.01 ml in one test, and 0.01 ml and 0.02 ml in the second. Negative controls were 0.1 mg discs of kanamycin (test-1) and 0.001 ml commercial bleach (test-2), substances known to be toxic, but not genotoxic, while the mutagens, ethylmethanesulfonate (EMS) and 1,2,3,4-diepoxybutane (DEB) at 0.001 ml each served as positive controls. [S-9 does not function in these disc assays, and was not included.]

In both experiments the test substance produced equal inhibition in cultures of both repair-deficient and normal strains, similar to those of the negative control chemicals, indicating bis (tri-N-butyltin) oxide apparently does not cause DNA damage requiring recombinational repair under the conditions employed for this assay.

EVALUATION: Since no data are presented indicating what concentrations of active ingredient were tested, the study is UNACCEPTABLE.

Copper-8-Quinolinolate (Caswell No. 253) Acc. # 248746 002581

(DER) DATA EVALUATION RECORD

FORMULATION: Reported as "active ingredient in Woodtreat®" [no other information given, but presumably received as a liquid].

CITATION: Microbiological Genotoxicity Assays of Copper-8-quinolinolate. (Report No. 3002-7, January, 1981, Genex Corp., Rockville, MD; sponsored by: Koppers Co., Pittsburgh, PA.)

TRADE SECRET CLAIM: CBI

REVIEWED BY: Irving Mauer, Ph.D. (HED/TB) Approved by:_____ (Date):_____

DATE OF REVIEW: February 26, 1983

TEST TYPES: Microbial Mutagenicity Assays: (A) Ames Salmonella/microsome Assay; (B) Escherichia coli WP2 (uvrA) Assay; (C) Saccharomyces cerevisiae D7 Assay; (D) Differential DNA Repair Assay with Bacillus subtilis H17/M45.

STUDIES: A. Salmonella typhimurium/Microsome (Ames) Assay.

Duplicate plate cultures of the histidine-requiring (his-) strains TA1535, TA1537, TA1538, TA98 and TA100 of S. typhimurium were exposed to graded concentrations (no less than 5) of test compound (in dimethylsulfoxide, DMSO) in replicate experiments, both in the absence and the presence of Aroclor 1254-induced rat liver homogenate metabolic activation system (S-9) prepared from adult Sprague-Dawley males, and the number of revertent colonies (his+) counted after three days incubation. Solvent (DMSO) and appropriate positive controls (bacterial mutagens) were included in each experiment.

In the first experiment (six doses ranging from 0.001 to 0.3 ul/plate), the two top doses (0.2 and 0.3 ul) were "toxic" [survival data, however, were not presented], and "small" non dose-related increases (2-3 x DM50 controls) were reportedly induced in TA1538 and TA100 in the presence of S-9 [NB: Table 1 also records comparable increases for non-activated cultures of TA1538 at "subtoxic doses," as well as smaller increases, 1.3-1.5X control in both activated and non-activated cultures of TA 98].

002581

The second experiment assayed a dose range of 0.001 to 0.2 ul in strains TA1535, TA1537 and TA98, 0.01 to 0.2 ul in TA100, but 0.0005 to 0.1 ul in TA 1538. "Small, non-reproducible" increases in revertents (1.4-1.8X control) were reported [in text only] with TA98 and TA100, but "not [in] the other strains at these doses" [NB: However, Tables 2 and 4 record comparable increases in TA1535 and TA1538, respectively.]

In the final experiment (7 doses, from 0.005 to 0.2 ul), small increases were observed for activated cultures of TA100 and TA98 [but not "dose related", as indicated in the text--see Table 5].

Positive controls responded as expected in all experiments.

The authors concluded that since copper-8-quinolinolate induced small [but not dose-related] increases in his+ revertents in strain TA100 in the presence of mammalian metabolic activation, the test compound may be considered a "weak mutagen that requires S-9 Mix."

EVALUATION: Despite the discrepancies between report text and tabulations [noted above], the reviewer tends to agree with the conclusion of the authors as to the mutagenicity (albeit "weak") of the test chemical, but would extend the positive result to also include TA1538 (both activated and not), and perhaps TA98, based upon the same criteria (indicated in the summary statement on p. 15). Though partly inconclusive, the study would be ACCEPTABLE, providing informaton on the nature (technical grade?; purity?) of the test substance and toxicity (survival data) were supplied).

B. Escherichia coli WP2 (uvrA) Assay.

Tryptophan-requiring cultures (tryp-) of the WP2 strain of E. coli, also deficient in excision repair (uvr-), were exposed to test compound in replicate experiments (duplicate plates at each dose), both in the presence and absence of an activated mixture of liver microsomes (S-9) from Aroclor 1254-induced adult male S-D rats, and revertent tryptophan-independent colonies (tryp+) enumerated after appropriate incubation. Positive controls (MNNG, 2 ug/plate; and 2-anthramine, 10 ug/plate) as well as the solvent (with or without S-9) were included in each experiment.

At dose ranges of 0.001 to 0.02 ul/plate (Table 6) or 0.0005 to 1.0 ul/plate (Table 7), no elevations in the number of tryp+ revertents were found. The top dose (1.0 ul) was said to be toxic [but no survival data were presented] All cultures responded as expected to the positive control mutagens.

The authors concluded that Copper-8-quinolinolate was not mutagenic under the assay conditions employed. 00258!

EVALUATION: The study would be ACCEPTABLE were information on the test substance (technical?; purity?), as well as survival (toxicity) data supplied.

C. Saccharomyces cerevisiae D7 Assay.

Three genotoxic endpoints were assayed in test compound-treated cultures of the dipoid yeast strain D7 of S. cerevisiae, which is an adenine heterozygote at two loci (ade2/ade2), and requires both tryptophan (tryp-) and isoleucine (iso-) for growth: Mitotic recombination (induction of aberrant, sectored, colonies); gene conversion (indicated by growth of tryptophan-independent colonies (tryp+), and reversion to isoleucine independence (iso+, either by reverse mutation at the same locus, or a non-specific suppressor mutation). Two separate experiments, each involving 4-hr incubations were conducted, both with and without added microsomes (S-9) prepared from the livers of Aroclor/254-induced adult male Sprague-Dawley rats. Both positive controls (MNNG, 0.0005% w/v, without S-9; sterigmatocystin, 0.25 or 0.05% w/v with S-9) and solvent (DMSO, with or without S-9) were included in both experiments. Triplicate cultures were treated at each dose of test substance, mutagen and solvent controls, and toxicity (% relative survival) determined throughout each experiment.

At doses of 0.0005 to 0.008% (v/v) test compound (test-1), dose-related increases in the number of recombinants were observed (3-5X DMSO controls) in non-activated cultures only at the moderately toxic (50% or less relative survival) higher doses (0.004, 0.006, 0.008%), but no increases in tryp+ convertents or iso+ revertants [Tables 8-10, Summary Table 11]. The second experiment (0.0005 to 0.004%, v/v) confirmed the positive recombinogenic result, yielding dose-related increases in aberrant colonies, but at lower concentrations, 0.001 through 0.003%, both in treated cultures without S-9 as well as in activated cultures. The highest dose here (0.004%) was lethal in non-activated cultures, and left only 4% survivors with S9. As in the first experiment, no increases were recorded in tryp+ conversion or iso+ reversion (Tables 12-14, Summary Table 15).

[Although not noted in the report text, the tabulations indicate poor performance of the positive control sterigmatocystin in the gene conversion and mutation portions of the study, 0.025% and 0.05% more often than not producing calculated responses no different from solvent controls.]

The authors concluded that copper-8-quinolinolate caused reproducible, dose related increases in aberrant colonies when tested without S-9, and [apparently] non-dose-related increases with S-9, and thus may be considered a recombinogen.

EVALUATION: Whereas the data for the recombination portion of the study has been generated by adequately controlled procedures, and thus supports the positive result obtained (Test compound is a recombinogen), (ACCEPTABLE), the portions meant to assay for gene conversion and mutation are inconclusive, mainly due to the inadequate response provided by the positive control, sterigmatocystin at the concentrations used (UNACCEPTABLE).

D. Differential DNA Repair (Toxicity) Assay with Bacillus subtilis.

Test compound-soaked paper discs were placed in the center of parallel cultures of the recombinational repair-deficient M45 (rec-) and normal H17 parental (rec+) strains of E. coli, and zones of growth inhibition compared (differential toxicity) in replicate experiments. [S-9 activation does not function in this type of assay]. Controls included kanamycin and commercial bleach (negative, since these substances are toxic, but not genotoxic), and the mutagens ethylmethanesulfonate and 1,2,3,4-diepoxybutane (as positive substances).

The test compound produced equal zones of inhibition in both strains (Tables 16, 17), and hence it was concluded that copper-8-quinolinolate was not genotoxic under the assay conditions used.

EVALUATION: Since no informaton is provided regarding the concentrations of test substance used, this study is UNACCEPTABLE.

3-Iodo-2 propynyl butyl carbamate. (Troysan) (Caswell No. 501A)

Acc. # 248747 002581

(DER) DATA EVALUATION RECORD

FORMULATION: WOODTREAT WB Concentrate, "a watery dark-brown liquid" [no other characteristics stated].

CITATION: Range Finding Toxicity Tests on Woodtreat WB Concentrate. (Report No. 44-516, May 27, 1981, Bushy Run Research Center, Export, PA; sponsored by Koppers Co., Pittsburgh, PA.)

TRADE SECRET CLAIM: CBI

REVIEWED BY: Irving Mauer, Ph.D. (HED/TB)

Approved by:_____
(Date):_____

DATE OF REVIEW: February 26, 1982

TEST TYPES: Acute Toxicity Tests: (A) Acute Oral LD50 - Rat; (B) Acute Dermal LD50 - Rabbit; (C) Acute Inhalation - Rat; (D) Acute Skin Irritation - Rabbit; (E) Acute Eye Irritation - Rabbit.

STUDIES:

A. Acute Oral LD50 in Rats

Groups of 5 each Wistar-derived juvenile male rats (3-4 weeks) were intubated with 4, 8 or 16 ml/kg undiluted test substance, and observed for 14 days.

Calculated LD50 (by moving-point average method) was 8.57 (5.8 to 12.7) ml/kg [TOX. CAT. IV]. Salivation, lacrimation and ataxia were seen within minutes in animals which died (1-2 days) after receiving the top dose; sluggishness, ruffled fur, discharges from nose and mouth as well as genital area within 24 hours of the 8 ml/kg dose; and minor salivation in only one rat at the low dose. Survivors to 14 days recovered within 4 days of dose, and weight gain was normal. Gas-filled (and stomachs) reddish intestines were found at gross pathological examination in dead animals, but no obvious changes in survivors. CORE - SUPPLEMENTARY.

B. Acute Dermal LD50 in Rabbits

Undiluted test material was applied to the clipped, intact trunk skin of young NZ white rabbits, 2 to 4 per

group, and retained for 24 hours under polyethylene sheeting; animals were observed for 14 days.

At doses above 1 ml/kg, all treated animals died within 1 to 3 days; 2 of 4 at 0.5 ml/kg, and 1 of 4 at 0.25 ml/kg. Erythema, edema and ecchymosis were noted at treated sites in all animals, low weight gain to weight loss in survivors of 0.5 ml/kg, and site scabs or fissuring at 14 days. [TOX. CAT. I]

Pathological examination in victims revealed mottled livers, dark spleens and white stomachs, but nothing remarkable in survivors. CORE SUPPLEMENTARY.

C. Acute Inhalation in Rats

Six animals [presumably young S-D males, but not stated] were exposed for 6 hours under static conditions in a sealed Plexyglas chamber to a "substantially saturated vapor" (fan-generated, spontaneous volatilization), and observed thereafter for 14 days.

No rats died, but weight gain was significantly reduced, and unsteady gait and lacrimation noted within one hour of removal from chamber, prostration and dyspnea within 4 hours [number unspecified]. CORE-SUPPLEMENTARY (no LC50 calculated, and static exposure does not meet Agency requirements).

D. Acute Skin Irritation in Rabbits.

0.01 Ml of undiluted test material, or diluted to 10% in water, was applied to the clipped, intact belly skin of 5 rabbits [presumably male NZ whites, but not stated], and held in place for 4 hours by a patch (DOT procedure).

In those animals treated with undiluted test substance, marked "capillary injection" was noted in four animals and necrosis in a [further?] one; while capillary injection and moderate erythema were found in four and one, respectively, treated with the same amount of the 10% agueous dilution. [Grade assigned in report was "6"; no PIS given]. The report "Results" stated: "2 of 2 rabbits with necrosis; therefore, 'corrosive material.'" [TOX. CAT. I]. CORE-SUPPLEMENTARY.

E. Acute Eye Irritation in Rabbits

0.005, 0.02, 0.1 or 0.5 Ml of undiluted test material was instilled into the conjunctival sacs of 5 rabbits, and read immediately, as well as after fluorescein staining 24 hour later.

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Moderate corneal "injury" [=opacity?] was observed in all eyes treated with 0.02 ml test material, accompanied by iritis in 2 eyes. No corneal (or other ocular) changes were noted in eyes instilled with the 0.005 ml dose. [Grade assigned by authors was "5"; no PIS given; presumably TOX. CAT. II]. CORE-SUPPLEMENTARY.